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A Review And Risk Assessment Of Toxic Cyanobacteria In The Hawke's Bay

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A Review And Risk Assessment Of Toxic Cyanobacteria In The Hawke's Bay

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EXECUTIVE SUMMARY

Cyanobacteria (blue-green algae) can produce natural toxins known as cyanotoxins. Cyanotoxins are a threat to human and animal health when consumed or through contact. Recent studies have shown that cyanotoxins are more prevalent in New Zealand water bodies than previously thought, especially when cyanobacterial blooms or mats are present.

There is a paucity of knowledge on the risks cyanobacteria pose in the Hawke's Bay region. In 2005, multiple dog poisonings occurred after swimming in Poukawa Stream. Sample analysis linked these poisonings to high concentrations of cyanobacteria and associated cyanotoxins. This event and other recent cyanobacterial blooms in recreational lakes have raised concerns regarding the potential health risks posed by cyanobacteria. Cawthron was asked to review the current data on the occurrence of cyanobacteria in lakes and rivers in the Hawke's Bay region. This data was used to identify areas of high risk, highlight knowledge gaps and suggest monitoring options that may lead to improved management of cyanobacteria in this region.

The review of the Hawke's Bay Regional Council's State of the Environment periphyton data showed that cyanobacteria were common in samples from 36 rivers. Of concern was the presence of *Phormidium* in the majority of samples. Species from this genus are known to produce cyanotoxins in New Zealand. Hawke's Bay rivers should be monitored for extensive mats of benthic cyanobacteria, particularly in areas of high recreation use or where stock and domestic animals have access. When mats are observed they should be examined microscopically and if potentially toxic species are observed, the presence of cyanotoxins should be investigated.

Problematic cyanobacteria have been recorded in the following lakes in the Hawke's Bay region: Eland, Hatuma, Oingo, Orakai, Opouahi, Runanga, Tutira, Whakaki (Lagoon) and Waikopiro. With the exception of Lake Waikaremoana and Lake Tutira, recent water quality and phytoplankton data for most Hawke's Bay lakes is deficient. A water quality survey of small lakes in the region was conducted in the mid 1980s, but subsequent monitoring was intermittent or non-existent. We recommend that a complete and comprehensive data set is collected and maintained for Hawke's Bay lakes. Lakes that are used for recreation and are known to experience regular cyanobacterial blooms should be monitored regularly throughout the year.

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1. INTRODUCTION

Cyanobacteria (commonly known as “blue-green algae”) are an ancient group of photosynthetic prokaryotic organisms found worldwide in diverse environments including oceans, freshwater, bare rock and soil (Whitton & Potts 2000). In aquatic environments, most cyanobacteria species occur in low concentrations however, when environmental and hydrological conditions are favourable, cyanobacteria cells can multiply and form algal blooms. Cyanobacteria can also grow on the bottom substrates of waterbodies, sometimes forming mats (benthic cyanobacteria) which can detach and form thick floating masses.

There are approximately 2000 cyanobacteria species worldwide and an increasing number of these are known to include toxin-producing strains. These natural toxins are a threat to human and animal health when consumed or through contact with them. The mechanisms of toxicity for cyanotoxins are very diverse, ranging from hepatotoxicity and neurotoxicity, to dermatotoxicity. Some cyanotoxins have also been shown to promote liver tumour growth when ingested in low doses over extended periods (Falconer & Humpage 1996; Chorus & Bartram 1999). Based on their chemical structure, cyanotoxins can be divided into three broad groups; cyclic peptides (microcystins and nodularins), alkaloids (cylindrospermopsins, anatoxins and saxitoxins) and lipopolysaccharides (LPS) (Table 1).

Until recently, knowledge of the occurrence of cyanotoxins and species responsible for cyanotoxin production in New Zealand was limited. However, recent studies have revealed the widespread and complex nature of contamination of our water resources by toxic cyanobacteria (Wood 2005; Wood et al. 2006a). Cyanobacteria species in New Zealand are known to produce the cyanotoxins: microcystins, nodularin, anatoxin-a, homoanatoxin-a, cylindrospermopsin, deoxycylindrospermopsin and saxitoxins (Table 1) (Wood & Stirling 2003; Wood et al. 2006a, b, c, d).

Table 1. Summary of the known cyanotoxins, their primary toxicological target in mammals, and known cyanobacteria genera that produce each toxin (adapted from Chorus & Bartram 1999). Toxins in bold type are known to occur in New Zealand. Genera in bold type are known to produce the associated toxin in New Zealand. *The results of cyanotoxin testing on environmental samples indicate that species from this genus produce the associated cyanotoxin in New Zealand (Wood & Stirling 2003; Wood 2005; Wood et al. 2006a, b, c, d).

Toxin Group and Toxin	Primary Target in Mammals	Cyanobacteria Genera
Cyclic Peptides		
Microcystins	Liver	***Microcystis, Anabaena*, Planktothrix*, Nostoc,*** *Haphalosiphon, Anabaenopsis, Aphanocapsa, Aphanizomenon, Arthrospira, Cylindrospermopsis,* ***Oscillatoria*, Phormidium*,*** *Pseudanabaena, Snowella, Synechocystis, Woronichinia.*
Nodularin	Liver	***Nodularia***
Alkaloids		
Anatoxin-a	Nerve synapse	***Anabaena*,*** *Planktothrix,* ***Aphanizomenon,*** *Cylindrospermum, Lyngbya, Microcystis,* ***Oscillatoria*,*** *Planktothrix,* ***Phormidium*,*** *Raphidiopsis.*
Homoanatoxin-a	Nerve synapse	*Planktothrix, Raphidiopsis,* ***Oscillatoria****, **Phormidium***,
Anatoxin-a(S)	Nerve synapse	*Anabaena, Oscillatoria.*
Cylindrospermopsins	Liver	***Cylindrospermopsis*,*** *Aphanizomenon, Umezakia, Anabaena, Raphidiopsis.*
Saxitoxins	Nerve axons	***Anabaena****, Aphanizomenon, Lyngbya, Cylindrospermopsis,* ***Planktothrix****.*
Lipopolysaccharides (LPS)		
LPS	Potential irritant; any exposed tissue	All

Cyanobacterial blooms are becoming a common seasonal phenomena occurring throughout New Zealand in a range of freshwater bodies used for drinking-water supplies, recreation and stock-watering (Wood 2005). The frequency of blooms and their geographic spread is likely to increase with the continuing eutrophication of New Zealand's freshwater, and global climate change. Benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers and are found in a wide range of water quality conditions, including relatively unenriched waters used for drinking-water supplies (Biggs & Kilroy 2000). Although benthic taxa have received less attention than their planktonic counterparts, they are increasingly being recognised as problematic as they are also known to produce toxins and taste- and odour-causing metabolites (Edwards et al 1992; James et al 1997; Mez et al 1997).

The Hawke's Bay region has not been exempt from cyanobacterial blooms. Lakes Tutira and Orakai have a long history of cyanobacterial blooms. Catchment management and bio-manipulation have previously been used to reduce cyanobacterial blooms in the Hawke's Bay region. Cyanobacteria are also a common component of periphyton samples collected from Hawke's Bay rivers as part of the "State of the Environment" monitoring programme. A recent dog poisoning event that was linked to cyanobacteria has highlighted the need for the Hawke's Bay Regional Council to become proactive in increasing their knowledge of cyanobacteria in the region. Cawthron was asked by the Hawke's Bay Regional Council to review existing data on the occurrence of planktonic and benthic cyanobacteria in the regions waterbodies. Using this information and water quality data, risks are discussed. Recommendations for management are made and important data gaps are highlighted.

2. BENTHIC CYANOBACTERIA IN THE HAWKE'S BAY REGION

Cawthron has undertaken the analysis of periphyton samples for the Hawke's Bay Regional Council as part of their "State of the Environment" (SoE) monitoring programme since 2003. Samples have been analysed from 36 different rivers. Some rivers have been sampled at more than one location (Table 2). A total of 165 river sites have been analysed during this period.

Table 2. Rivers in the Hawke's Bay region that have been sampled as part of the "State of the Environment" programme and the number of sampling locations in each river.

River	No. of sampling locations	River	No. of sampling locations
Anaura	1	Porangahau	3
Aniwaniwa	2	Puhokio	1
Aropaoanui	1	Ripia	1
Esk	2	Ruakituri	1
Hangaora	1	Sandy	1
Kopuawhara	1	Taharua	3
Mahiaruhe	1	Te Iringawharo	1
Makaretu	1	Te Kumi	1
Mangakuri	1	Tuataekuri	1
Manganouku	1	Tuki Tuki	3
Mangaone	1	Tukipo	1
Mangaonuku	1	Tutaekuri	2
Mangaorapa	1	Waiau	2
Mangatarata	3	Waikaretahekei	2
Mohaka	2	Waingongoro	1
Ngaruroro	7	Waipawa	1
Opoutama	1	Wairua	1
Pohoukio	1	Waitio	1

For each sample the relative abundance estimates are recorded for different micro-algae species observed. Relative abundance estimates are of most use for identifying the dominant taxon/taxa in a sample and providing a list of the taxa that are present. These estimates do not provide information about cell concentrations or overall algal biomass. Therefore the relative abundances could not be compared between sampling locations or between different sampling dates at one location. No information was provided on how the samples were collected.

Seventeen different cyanobacteria genera were observed in the periphyton samples. Several species were also observed that could not be attributed to a genus but were obviously from the Nostocales order (Table 3). *Phormidium* was the most commonly observed genera occurring in 56% of samples. It was also often the most abundant genera in each sample. Species from this genera were recently found to produce anaotoxin-a and homoanatoxin-a in samples collected from rivers in the Wellington region (Cawthron, unpublished data). *Heteroleibleinia* was the second most abundant genera, observed in 47% of samples. *Heteroleibleinia* is a small epiphytic cyanobacterium which is commonly found attached to other algae. Six of the genera observed in the samples contain species known to produce cyanotoxins. Species from the order Nostocales also produce cyanotoxins (Table 3).

Table 3. Cyanobacteria genera observed in Hawke's Bay periphyton samples and the percentage of samples each genera was observed in. Genera and order highlighted in yellow contain species known to produce cyanotoxins .

Genera	% of samples genera present
Anabaena	4%
cf. *Calothrix*	1%
cf. *Tolypothrix*	1%
Chamaesiphon	34%
Chroococcus	1%
Coleodesmium	1%
Geitlerinema	1%
Heteroleibleinia	47%
Leptolyngbya	5%
Lyngbya	6%
Merismopedia	12%
Nostoc	5%
Oscillatoria	5%
Phormidium	56%
Planktolyngbya	2%
Pseudanabaena	10%
Spirulina	5%
Order	
Nostocales	4%

Cyanobacteria were observed in all rivers and on almost every sampling occasion. Cyanobacteria were not detected in 4% of the samples. Cyanobacteria diversity was usually restricted to 1-3 genera per sample (89%), with 4-7 different genera observed in only 7% of samples.

These results demonstrate that cyanobacteria (both known toxic and non-toxic genera) are an integral component of Hawke's Bay rivers. In low abundances most cyanobacteria will not be problematic. Without quantitative data, no conclusions on cyanobacteria biomass and associated problems in these rivers can be made. Cyanobacterial biomass should be monitored in areas which have high recreational use or in areas where domestic animals or stock have access. Lamason et al. (2006) identified four river sites as important recreation areas; Eskdale Park on the Esk River, Ngaruroro River at the Chesterhope Bridge, Tutituki River at Waipukukurau and Tutaekuri River at the Pakowhai Road Bridge. These rivers are all monitored as part of the Hawke's Bay SoE monitoring programme. Macroscopic observation of the extent of mat coverage could be made at sites and transects across rivers taken if there is cause for concern. Macroscopic observations should be investigated by microscopic analysis.

2.1. Factors promoting benthic cyanobacterial growth

Bergey (1998) carried out a study investigating the environmental factors associated with periphyton biomass in Hawke's Bay rivers. Although no cyanobacteria were observed in this study, it provides some valuable information of the relationships between environmental factors and periphyton biomass. Conductivity (as a measure of nutrient supply) and mean velocity of river flows were the main factors associated with chlorophyll concentration.

There is currently little information available on environmental and water quality factors that lead to benthic cyanobacteria proliferation. A recent incident in the Hutt River (Lower Hutt) suggests that periods of low and stable flow can result in benthic cyanobacterial growth. Unusually low and stable flows in the Hutt River during October and November 2005 resulted in the proliferation of benthic cyanobacteria which formed large black/brown mats along the river edge (Figure 1). At least five dogs died during November after swimming in the river. Analysis of the stomach contents of one dog and cyanobacterial mats found at the site revealed *Oscillatoria* filaments and two neurotoxic cyanotoxins, anatoxin-a and homoanatoxin-a. This case highlights the potential risks of benthic cyanobacteria.

Care should be taken before altering flow regimes of rivers which may result in extended periods of low and/or stable flows. Further work is required before the variables promoting benthic cyanobacterial growth in New Zealand rivers are understood.

Figure 1. Detached benthic mats on the edge of the Hutt River.

3. PLANKTONIC CYANOBACTERIA IN THE HAWKE'S BAY REGION

Four lakes in the Hawke's Bay region have been identified by the Hawke's Bay Regional Council as being of major significance; Lake Waikaremoana, Lake Waikare-iti, Lake Tutira and Lake Poukawa (HBRC 2005, see Appendix 1). Lakes Waikaremoana, Waikare-iti and Tutira all have high recreational use. Waikaremoana and Waikare-iti are situated close together within a largely undisturbed catchment of mostly native forest. Both lakes are deep, monomictic lakes that have been classified as oligotrophic in the past. However, water quality data for Lake Waikare-iti is lacking.

Lake Tutira is also a deep monomictic lake and is situated within a pastoral catchment which is now a Department of Conservation wildlife refuge. As a result of converting the lake catchment into pastoral land, Lake Tutira has suffered from severe eutrophication. Cyanobacterial blooms (*Anabaena* and *Microcystis* spp.) have been recorded in the lake since 1972. These blooms have impacted on its use for swimming, fishing, boating and camping (McColl 1978). *Anabaena* and *Microcystis* spp. are known to produce a range of cyanotoxins internationally.

Lake Poukawa is situated within an intensely farmed area and is an extremely shallow lake (maximum depth 1 m). Recreational use for Poukawa is less than the other major lakes due to the catchment being privately owned. However, there is stock access and suspected cyanobacterial poisonings have occurred in adjacent areas (see Section 4). This lake has been classified as eutrophic but because of extensive areas of macrophytes, visible cyanobacterial blooms have not been recorded in the past (Hooper 1987).

An additional four lakes have been identified as being of significant value (HBRC 2005, see Appendix 1); Whakaki Lake (Lagoon), Lake Oingo, Lake Hatuma and Lake Runanga. These lakes are all privately owned and have all experienced cyanobacterial blooms. Whakaki Lake is an important wetland used for hunting and fishing and is currently being managed by the Whakaki Lake Trustees. Limited data exists for this waterbody but blooms of potentially toxic cyanobacteria species have been recorded in the lake. The other three lakes all have limited public or stock access. Lake Oingo experiences seasonal blooms of *Microcystis* spp. and extensive mats of the gelatinous species *Aphanothece stagnina.* Benthic *Aphanothece* spp. have recently been associated with cyanotoxin production (Dasey et al. 2005). Both Lakes Hatuma and Runanga have been classified as eutrophic lakes with cyanobacterial blooms detected previously. The only major survey of water quality for these lakes was conducted in the mid 1980s (Hooper 1987).

A number of other small lakes exist in the Hawke's Bay region; however water quality data for these lakes is either out-dated or non-existent. Lakes Waikopiro and Orakai are in close proximity to Lake Tutira and are within the same wildlife reserve. Both lakes are also known to experience cyanobacterial blooms and receive a moderate amount of recreational use. Lake Orakai was used to trial the use of silver carp to control cyanobacterial blooms (Carruthers 1986) and was considered one of the most eutrophic lakes in New Zealand at the time. Lake Opouahi is also situated within a reserve with significant recreational use especially fishing. It is a small but relatively deep lake and had been classified as oligotrophic to slightly mesotrophic in 1987 (Hooper 1987).

With the exception of Lake Waikaremoana and Lake Tutira, recent water quality data for most Hawke's Bay lakes is deficient. The Hawke's Bay Catchment Board conducted a water quality survey of small lakes in the region in the mid 1980s (Hooper 1987) but monitoring after this study was intermittent. Lake Tutira is the only lake that is currently being monitored specifically for the risk of cyanobacterial blooms. Lake Waikaremoana has been the subject of a number of water quality studies and results indicate that this lake is in relatively good condition with high water quality and clarity (Schwarz et al. 1991). However, regular monitoring of such an important waterbody in order to detect any long term changes in water quality is recommended.

After assessing the potential risk of cyanobacterial bloom formation against the recreational use of the most significant lakes in the Hawke's Bay Region (Appendix 1) it is recommended that the following lakes are regularly monitored for cyanobacteria; Lake Tutira, Whakaki Lake (Lagoon) and Lake Waikopiro. We would also recommend that a baseline survey be conducted on Lakes Waikaremoana, Waikare-iti, Poukawa, Opouahi, Orakai and Hatuma to assess the current situation as data is limited and these lakes have moderate to high recreational use. Lakes Opouahi, Orakai and Hatuma have all been recorded as experiencing cyanobacterial blooms in the past (Hooper 1987).

3.1. Lake Tutira

Lake Tutira is a small deep (maximum 42 m) lake situated in the Aropaoanui River Catchment (Hooper 1989). It is an important recreational area for the Hawke's Bay region and has been classified as eutrophic since the 1960s. In order to prevent further deterioration, Lake Tutira was the subject of the first major lake restoration project in New Zealand (McColl 1978). To reduce phosphorus inputs into the lake, the main inlet, Sandy Creek, was diverted from entering Tutira. The lake now only receives runoff from the immediate catchment. Regions of the lake edge have been fenced off from stock and riparian plantings were established (Hooper 1989). By the end of the 1980s Lake Tutira showed slight improvements in water quality and the lake remains categorised as eutrophic with limitations to recreational users and fisheries (Hooper 1989). In recent years health warnings have been issued for the lake as concentrations of potentially toxic cyanobacteria have exceeded levels safe for recreational contact (i.e. >15 000 cells/mL).

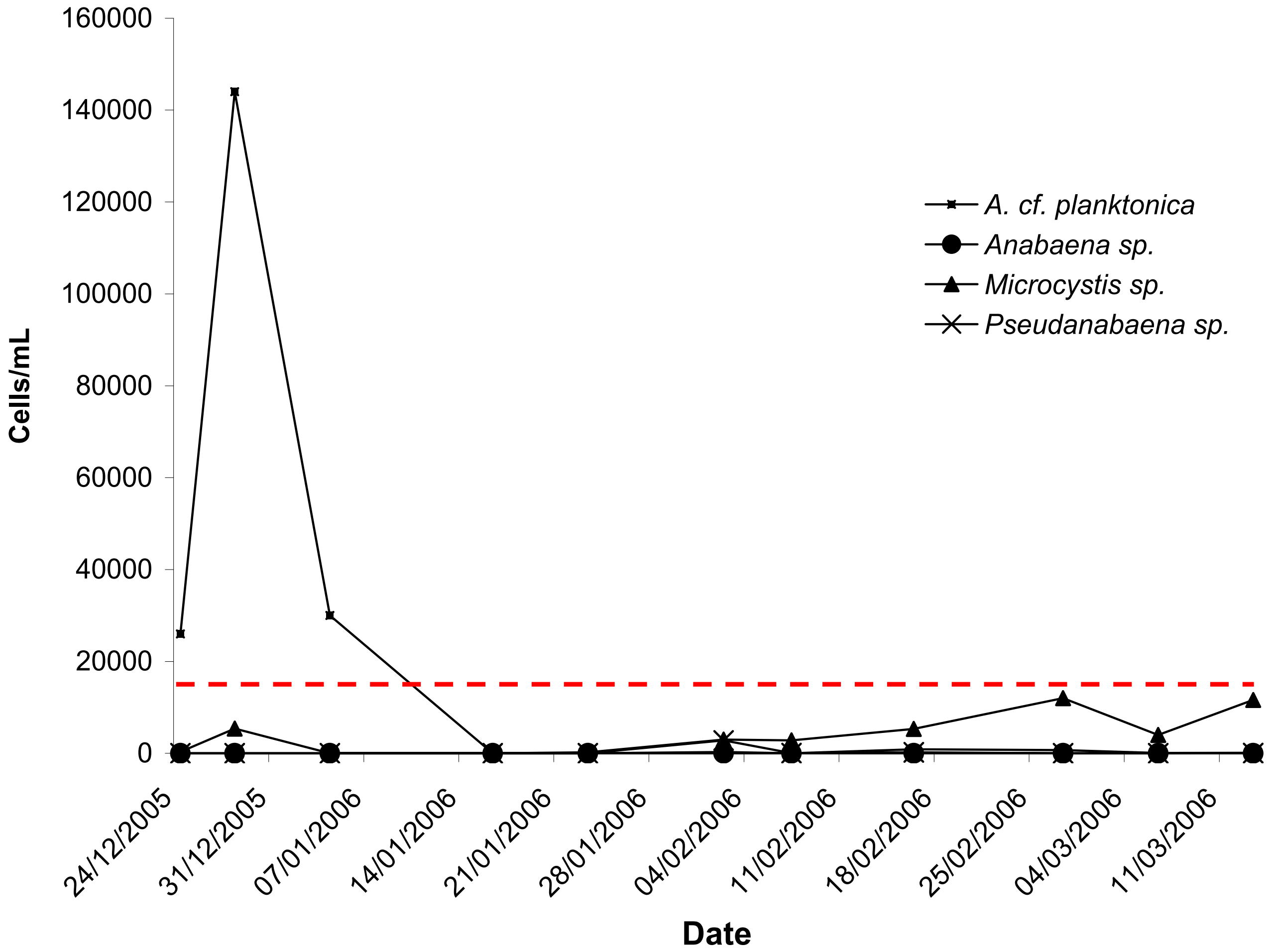

Figure 2. Cyanobacteria cell concentrations for Lake Tutira during the summer 2005/2006.

In 2005/2006, a dense bloom of *Anabaena* cf. *planktonica* formed in Lake Tutira, prompting regular monitoring. Samples were collected weekly and phytoplankton composition assessed by Cawthron's Microalgae Laboratory. *Anababena* cf. *planktonica* dominated in December 2005, and early January, 2006. Concentrations dropped substantially in late January and total cell concentrations remained below 15 000 cells/mL for the remainder of the sampling period (Figure 2). *Microcystis* spp. concentrations increased in February and although remaining below the 15 000 cells/mL threshold should have prompted concern as this species is commonly associated with microcystin production in New Zealand (Wood et al. 2006). To date there is no conclusive evidence that *A*. cf. *planktonica* produces known cyanotoxins in New Zealand.

3.2. Recreational Guidelines for cyanobacteria

New Zealand is currently looking to adapt the "Cyanobacteria and Algae in Freshwater" section from the "Australian Guidelines for Managing Risks in Recreational Water" (NHMRC 2005). The guidelines are based on cell counts and biovolumes of potentially toxic cyanobacteria, and cyanotoxins. The guidelines suggest a three-tiered system for alert levels in recreational freshwaters. Recreational freshwaters that exceed:

- 10 µg/L total microcystins; or ≥50 000 cells/mL toxic *M. aeruginos*a; or biovolume equivalent of ≥4 mm^3/L for the combined total of all cyanobacteria where a known toxin producer is dominant in the total biovolume; or
- 10 mm^3/L for total biovolume of all cyanobacteria material where known toxins are not present,

are assigned Red Alert Level – Action Mode. Local and health authorities then must notify the public that the water body is considered to be unsuitable for recreational contact. Monitoring should increase to weekly sampling or twice weekly if there is urgent need to determine when the bloom is in decline so that normal activities can resume. Alert levels may be downgraded once two successive representative samples show a decreasing trend below the higher alert level.

3.3. Proposed cyanobacteria recreational monitoring regime for Lake Tutira

Sampling sites for Lake Tutira should include shoreline areas that are regularly used for recreation as well as any areas with surface scums identified during site inspections. Samples should preferably be pooled to reduce spatial variability. We recommend that recreational monitoring for cyanobacteria in Lake Tutira follows a three-tiered warning system adapted from the Australian Guidelines for Managing Risks in Recreational Water 2005 (Table 4). This system is based on biovolume measurements rather than cell counts as biovolume takes into account the variability in size of cyanobacteria in a sample. Cell counts can bias the result when there are high numbers of small sized cyanobacteria as toxin content is more closely related to biovolume than total cell number. Using biovolume rather than cell counts may reduce the unnecessary issuing of health warnings.

During the months April to October, monthly sampling should be sufficient, with visual inspections conducted on a fortnightly to weekly basis. Samples should be taken at least two weeks before any major recreational event at the lake. Additional information that may help predict bloom development include; nutrient concentrations (e.g. nitrogen and phosphorus), temperature and dissolved oxygen profiles, and transparency (e.g. Secchi disc depth). Samples for nutrient concentrations generally need to be taken less frequently than samples for cyanobacteria concentrations as they have lower seasonal variability or may always be too high to limit cyanobacterial growth (Chorus & Bartram 1999). Monthly sampling for nutrient concentrations is best done at the beginning of a monitoring programme until long term trends are established and a reduction in sampling may be reasonable. If possible temperature and dissolved oxygen profiles as well as transparency should be measured on each sampling occasion. This will also provide valuable water quality information for this lake allowing comparison with existing data and baseline data for future changes in the water quality of the lake.

If cell concentrations begin to increase or when recreational use is high (e.g. from November to March) samples should increase to fortnightly or weekly (Amber Alert Level, Table 4). When cyanobacteria or cyanotoxin concentrations exceed the levels for Red Alert Level, local authorities should notify health authorities and the public that the lake is unsuitable for recreational contact. This can be done by erecting warning signs near areas of high use (Figure 3) and by issuing health warnings in local publications. It is also advisable to test for cyanotoxins once the waterbody reaches Red Alert Levels. Health warnings can be downgraded once two successive samples (usually collected one week apart) show concentrations below the alert levels.

Table 4. Suggested alert levels and monitoring programme associated with cyanobacteria for Lake Tutira. Adapted from Australian Guidelines for Managing Risks in Recreational Water" (NHMRC 2005).

Alert Level	Cyanobacteria Levels	Monitoring Requirements
Green	>500 to <5 000 cells/mL *Microcystis* spp. or biovolume equivalent of >0.04 to <0.4 mm^3/L for the combined total of all cyanobacteria.	Monthly sampling for cyanobacteria concentrations. Regular visual inspections for changes in water discolouration or scums are advised.
Amber	>5 000 to <20 000 cells/mL *Microcystis* spp. or biovolume equivalent of >0.4 to <4 mm^3/L for the combined total of all cyanobacteria	Increase sampling to fortnightly or weekly monitoring. Waterbody may be downgrade to Green Alert Level once two successive representative samples show a decreasing trend below the Amber Alert Level.
Red	10 µg/L total microcystins; or >20 000 cells/mL *Microcystis* spp.; or biovolume equivalent of ≥4 mm^3/L for the combined total of all cyanobacteria where a potential toxin producer is dominant in the total biovolume; or 10 mm^3/L for total biovolume of all cyanobacteria material where known toxins are not present.	Weekly monitoring. Local authorities and health authorities to warn the public that the lake is considered unsuitable for recreational contact. Cyanotoxin testing is recommended. Waterbody may be downgrade to Amber Alert Level once two successive representative samples show a decreasing trend below the Red Alert Level.

Figure 3. Examples of health warning signs for cyanobacteria at freshwater lakes.

4. SAMPLING FOR CYANOBACTERIA AND CYANOTOXINS

4.1. Phytoplankton

When monitoring a waterbody for cyanobacterial blooms it is important that the measurements of cyanobacteria or cyanotoxin concentrations are representative of the whole area. The recommended sampling procedure for recreational waters is to collect a single sample or a pooled sample for each recreational site in the waterbody (NHMRC 2005). The pooled sample comprises of five subsamples collected randomly within an area and mixed in a single container (e.g. a bucket) from which the pooled sample is taken for the cell count or cyanotoxin analysis. Sampling by this method accounts for spatial variability within a site. It is also recommended that additional samples are taken when obvious. Information that should be recorded is temperature, wind speed and direction, and weather conditions including the conditions 24 hours previously. The assessment should be on the areas most likely to be affected (e.g. downwind shores) and any visible scums or accumulations should be sampled as these represent the maximum hazard at the time the samples are collected. Note however that not all cyanobacteria form scums or obvious blooms e.g. *Cylindrospermopsis*. Such species can be present in high concentrations but may not be visually noticeable. Samples for cyanotoxin analysis should be collected in the same manner as for cyanobacteria. Generally, at least 500 mL should be collected preferably in a glass container. Sample for cyanotoxins should not be preserved but kept cool and in the dark till analysis. Samples for cyanobacteria should be preserved with Lugol's solution as soon as possible; it is often useful to keep part of the sample unpreserved for identification purposes.

4.2. Benthic Cyanobacteria

Benthic cyanobacterial mats are usually dark brown/black. This can change with differing flow regimes and species present. Figures 4A - F shows the appearances of the most common toxic benthic cyanobacterial mats in New Zealand.

Samples of benthic cyanobacteria can be collected by scraping or collecting mats and placing in jars/bottles. Samples should be stored chilled and in the dark and sent immediately for microscopic analysis. Lugol's preservative can be used to prevent sample degradation however **do not** preserve the entire sample as cyanotoxin analysis cannot be undertaken on Lugol's preserved samples. If the sample cannot be sent immediately, store the Lugol's preserved samples in the dark and freeze the remainder of the sample.

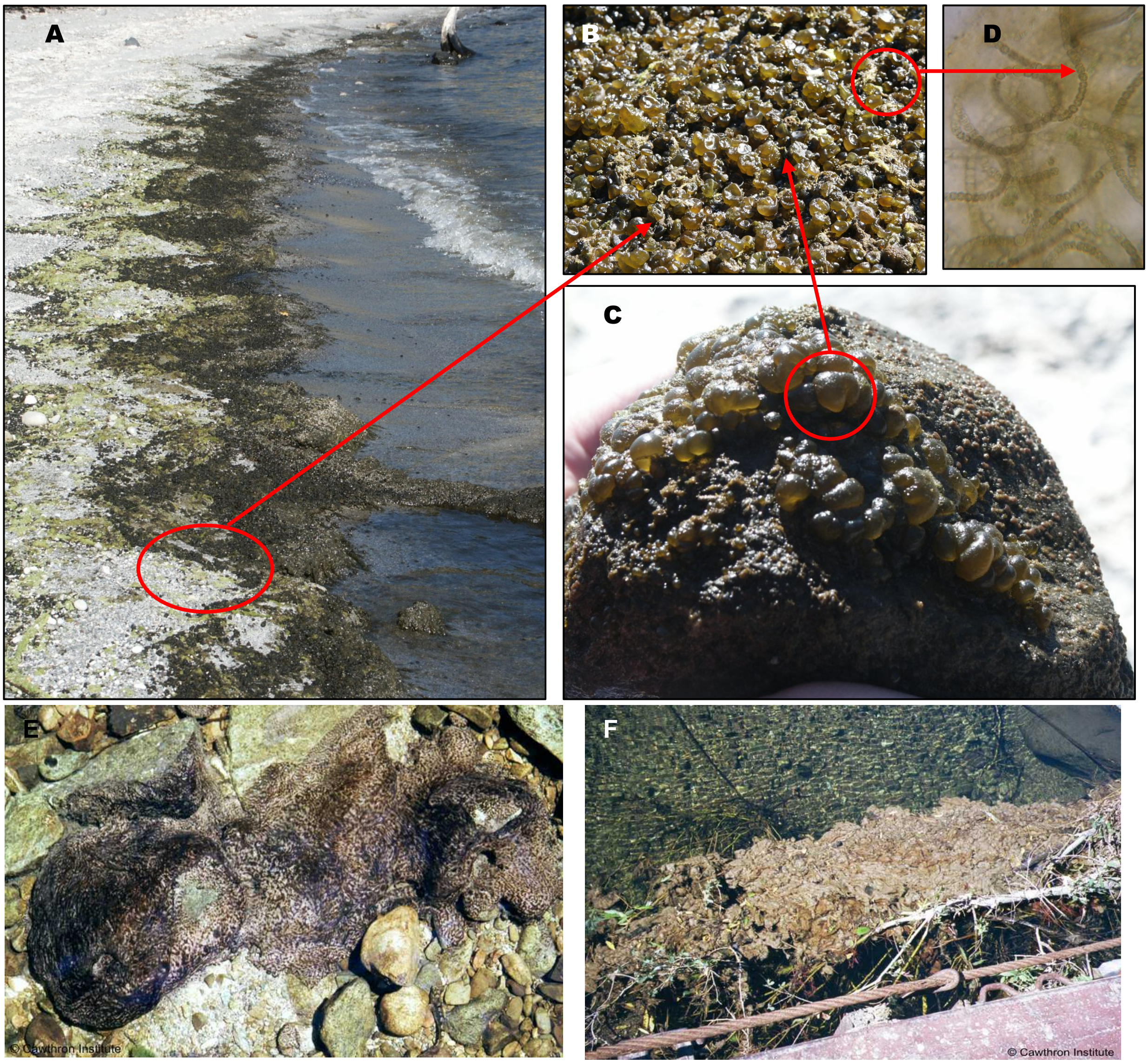

Figure 4. A, B *Nostoc commune* mats along the shores of Lake Taupo; C Low lake levels and high winds caused *N. commune* colonies to be dislodged from rocks in the lake and accumulate along the shoreline; D *N. commune* filaments under the microscope. High levels of microcystins were recorded in these mats E. Benthic mat of *Phormidium* sp. attached to river substrate F. Detached benthic mat accumulating along the edge of a river.

5. RECENT POISIONING EVENT

5.1. Description of Event

In August 2005, two dogs swimming in Busby Dam (near Poukawa Hastings) (Figure 5) suffered paralysis on two separate occasions. In both incidences after seeking veterinary assistance the dogs recovered. The stream below the dam is used for watercress collection and thus there was concern over potential human health risks. Cawthron was contracted by the Hawke's Bay District Health Board under the Ministry of Health "Supporting Local Needs Programme" to identify potentially toxic cyanobacteria species and determine if cyanotoxins were present in water samples from the dam and stream.

Figure 5. Busby Dam. Photo: S. Halliday (Hawke's Bay Regional Council).

5.2. Results

5.2.1. Species Identification

The sample from the dam contained large quantities of *Azolla* (water fern). A potentially toxic cyanobacteria species was identified in two samples; Busby Dam (05PF0339:31/08/050) and Stream (05PF0341:31/08/05). The species was identified as *Oscillatoria* cf. *sancta* (Figure 6). The *Oscillatoria* species appeared to be living predominately amongst the *Azolla* roots.

Species identification and cell counts were undertaken on further samples collected at the same site and nearby sites on 30/9/05.

Busby Dam 05PF0360:27/09/05 - One potentially toxic species of cyanobacteria was detected in the sample at low abundance.

Poukawa Stream 05PF0363:27/09/05 - Three potentially toxic species of cyanobacteria were detected in the sample.

Wetland 2 05PF0366:27/09/05 - No cyanobacteria were detected in the sample.

Poukawa 2 05PF0364:27/09/05 - Three potentially toxic species of cyanobacteria were detected in the sample.

Neighbours Dam 05PF0365:27/09/05 - Three potentially toxic species of cyanobacteria were detected in the sample.

Busby Dam 2 05PF0361:27/09/05 - Three potentially toxic species of cyanobacteria were present in the sample at high abundance. Cell counts were undertaken on this sample. *Oscillatoria* cf. *sancta* had the highest concentration (69 000 cells/ml) with two smaller Oscillatoria species also present; *Oscillatoria* sp. A (27 000 cells/mL) and *Oscillatoria* sp. B (54 000 cells/ml).

Figure 6. *Oscillatoria* cf. *sancta*

5.2.2. Cyanotoxin analysis

The sample collected from Busby Dam on 31/8/05 was analysed for the cyanotoxins; microcystins, nodularin, anatoxin-a, cylindrospermopsin and saxitoxins (Table 5). All results were negative. To assess the toxicity of the sample a mouse bioassay was also carried out using an extract from this sample. Three mice died within five minutes, indicating the presence of a neurotoxin. The same sample was then prepared identically and also split into three fractions (non-polar, organic and polar). No toxicity was detected in any fraction or in the identically prepared sample. A concentrated sample of *Oscillatoria* cf. *sancta* was prepared by micro-pipetting from the stream sample, no toxicity was observed in this sample. It is plausible that the lack of toxicity in the second set of testing was due to sample degradation (unlikely as samples were freezer stored) or that the toxin levels were lower than in the initial sample and were not at sufficient levels to see any observable effects in the mice.

Table 5. Toxin/toxicity analysis results.

Sampling Site	Sample Date	Sample Code	Laboratory ID	Toxin	Result
Busby Dam (Hawke's Bay)	31-08-05	91149	P51703-7	Saxitoxins[1]	Not detected
				Microcystin[2]	Not detected
				Microcystin[3]	Not tested
				Anatoxin-a[4]	Not detected
				CYN[4]	Not detected
				Toxicity[5]	**Toxicity detected**
				Toxicity[6]	Toxicity not detected
				Toxicity[7]	Toxicity not detected

1 Saxitoxins by Jellet Rapid test – an antibody based test.
2 Microcystin's and nodularin by LCMS. Calibration based on response for LR.
3 Microcystin activity by protein phosphatase inhibition. Calibration based on response for LR.
4 Anatoxin-a, homoanatoxin-a and cylindrospermopsin (CYN) by LCMS. Some interferences were present for anatoxin-a.
5 Toxicity by mouse bioassay. A crude extract was injected into the intraperitoneal cavity of three mice and all died within four minutes indicated the presence of a potent neurotoxin.
6 Fractions were prepared and tested by mouse bioassay. No toxin was present in any fraction.
7 Concentrated sample of cyanobacteria cell collected. Toxicity by mouse bioassay. A crude extract was injected into the intraperitoneal cavity of three mice. No toxicity was observed.

5.3. Summary of event and recommendations

Due to the high levels of *Oscillatoria* cf. *sancta,* the symptoms of the dogs and initial toxicity results we recommend that regular monitoring should be undertaken, particularly in areas where the public may have access to this waterway. Owners who have land adjacent to this stream should receive information detailing the potential risks.

6. CONCLUSIONS AND RECOMMENDATIONS

Reported incidences of toxic cyanobacteria are becoming increasingly prevalent in New Zealand's freshwater. This is partly due to increasing eutrophication of waterbodies, however, it is also likely to be a result of higher frequency of sampling, growing public awareness of the potential health hazards and improving technologies for micro-algae/cyanobacteria and cyanotoxin identification. A recent dog poisoning event in the Napier region was linked to cyanobacteria and highlighted the need for the Hawke's Bay Regional Council to become proactive in increasing their knowledge of cyanobacteria in the region.

A review of the Hawke's Bay Regional Council's State of the Environment periphyton data identified genera known to produce cyanotoxins. *Phormidium* was prevalent in numerous samples. Species from this genus have recently been associated with homoanatoxin-a and anatoxin-a production in samples collected from rivers in Wellington. In low abundance, most benthic cyanobacteria are unlikely to be a significant health risk, however, cyanobacteria biomass should be carefully monitored particularly at sites which have high human or animal use. Additional periphyton samples, water quality and river flow data should be collected if extensive growths are observed. Further sampling and baseline studies would be required to establish if benthic cyanobacteria are problematic in Hawke's Bay rivers. Environmental parameters leading to accelerated growth of benthic cyanobacteria are poorly understood in New Zealand and require further research. Low and stable flows appear to be a key factor resulting in accelerated growth in some rivers.

Cyanobacteria are a common component of phytoplankton communities in Hawke's Bay lakes. Eight lakes have been recorded as experiencing blooms of known toxin-producing species and benthic mats of the potentially toxic *Aphanothece* sp. are common in Lake Oingo. There is a lack of water quality and phytoplankton data for lakes except Lakes Tutira and Waikaremoana. Further information is required to establish baseline data and accurately assess the current situation. Lake Tutira regularly experiences blooms of *Anabaena* sp. and *Microcystis* sp. This lake has been monitored semi-regularly and we recommend establishing a regular sampling programme for this lake in line with the protocol suggested in this report.

A dog poisoning event in August 2005 was linked to cyanobacteria ingestion. It is highly likely that such events will occur again. Management options to reduce or remove problematic species need to be multi-tiered and should be the result of thorough research into possible causes. In the interim, water users should be made aware of potential health risks.

In conclusion we recommend that further surveys of waterbodies are undertaken during high risk periods (e.g. summer months). This information can then be used as baseline data to assess current and future changes in water quality and identify areas of high risk. Once identified, these areas may require regular monitoring and management options could be explored.

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Appendix 1.

Lake	Size/max depth	Catchment type	Current use?	Trophic classification?	Chl a levels	Nutrient levels	Stratification?	Experienced blooms in the past? Dominant species?	References
Lake Waikaremoana	55.74 km2/248 m	Undisturbed catchment. Podocarp, beech, broadleaf forest	Multiple recreational use. Hydro-electric power.	Oligotrophic	mean levels approx. 0.7 mg/m3 (1991)	DRP <1 mg/m3, NH4 <10 mg/m3, NO3 >40 - <3 mg/m3	Monomictic	Diatoms and green algae	Christmas et al. 1995, Howard-Williams et al. 1991a, Schwarz et al. 1991, Vincent et al. 1991, Howard-Willams et al. 1991b, Livingston et al. 1986, Howard-Williams et al. 1986
Lake Waikare-iti	3.33 km2	Mostly native forest	Scenic reserve	----	----	----	----	----	Livingston et al. 1986, Jolly 1968
Lake Poukawa	2.25 km2/1 m	Pasture	Private land. Stock access.	Eutrophic	10.6 mg/m3 (1987)	TP = 0.15, TN = 2 (1987)	None	No, high levels of macrophytes	Hooper 1987
Lake Tutira	1.74 km2/42 m	Pasture	High recreational use (fishing, swimming, boating, camping)	Eutrophic	12 mg/m3 (1978)	TP = 0.02, TN = 0.5 (g/m3, 1987)	Monomictic	Yes, *Anabaena* and *Microcystis* spp.	Livingston et al. 1986, Hooper 1989, McColl 1978, HBRC raw data 05/06
Lake Oingo	0.86 km2/2.5m	Wetlands and pasture	Private land. Stock access.	Eutrophic	102 mg/m3 (1987)	TP = 0.22, TN = 3.6 (g/m3, 1987)	----	Yes, *Microcystis* and *Aphanothece*	Hooper 1987
Whakaki Lake (Lagoon)	----	----	Privately owned by Whakaki Lake Trustees. Hunting and fishing.	----	----	----	----	Yes, *Anabaena flos-aquae* and *Nostoc commune*	HBRC raw data 2002
Lake Hatuma	2.43 km2/1 m	Wetlands and pasture	Private land. Wildlife reserve? Areas of public access.	Eutrophic	43 mg/m3 (1987)	TP = 1.9, TN = 2.9 (g/m3, 1987)	----	Yes, cyanobacteria (species unknown)	Hooper 1987
Lake Runanga	1.7 km2/1 m	Wetlands and pasture	Private land. Stock access.	Eutrophic	67 mg/m3 (1987)	TP = 21, TN = 3.8 (g/m3, 1987)	----	Yes, cyanobacteria (species unknown)	Hooper 1987

Lake Eland	0.04 km2/7 m	Pasture, not outlet or inlet	Private land. Stock access. Used as a stock drinking water supply	Eutrophic	33 mg/m3 (1987)	TP = 0.05, TN = 1.5 (g/m3, 1987)	----	Yes, cyanobacteria (species unknown)	Hooper 1987, Sander 1994
Lake Orakai	0.04 km2/6 m	Pasture	Wildlife refuge.	Highly eutrophic	----	TP = 135-420, TN = 1360-2855 (mg/m3, 1986)	Monomictic	Yes, cyanobacteria spp. e.g. *Microcystis* sp.	Carruthers 1986
Lake Waikopiro	0.099 km2/18 m	Pasture	Situated within a reserve. High amount of recreational use e.g. walking, camping, boating, fishing	Eutrophic	17 mg/m3 (1987)	TP = 0.07, TN = 1.5 (g/m3, 1987)	----	Yes, *Anabaena* and *Microcystis* spp.	Hooper 1987
Lake Opouahi	0.063 km2/24 m	Mostly native forest, some pasture.	Situated within a reserve. High amount of recreational use e.g. walking, swimming, boating, fishing	Oligotrophic to slightly mesotrophic	3.4 mg/m3 (1987)	TP =<0.01, TN = 0.3 (g/m3, 1987)	Monomictic	Yes, but noted in 1987 that algal blooms had reduced due to increased catchment management.	Hooper 1987

(dark turquoise)	The four major lakes identified in the Proposed Hawke's Bay Regional Resource Management Plan (2005)
(light turquoise)	Other major lakes also identified in the Proposed Hawke's Bay Regional Resource Management Plan (2005)
(yellow)	Other significant lakes in the Hawke's Bay Region.